

Protein Inhalation Powders: Spray Drying vs Spray Freeze Drying

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Purpose. To develop a new technique, spray freeze drying, for preparing protein aerosol powders. Also, to compare the spray freeze-dried powders with spray-dried powders in terms of physical properties and aerosol performance.

Methods. Protein powders were characterized using particle size analysis, thermogravimetric analysis, scanning electron microscopy, X-ray powder diffractometry, and specific surface area measurement. Aerosol performance of the powders was evaluated after blending with lactose carriers using a multi-stage liquid impinger or an Anderson cascade impactor. Two recombinant therapeutic proteins currently used for treating respiratory tract-related diseases, deoxyribonuclease (rhDNase) and anti-IgE monoclonal antibody (anti-IgE MAb), were employed and formulated with different carbohydrate excipients.

Results. Through the same atomization but the different drying process, spray drying (SD) produced small ($\sim 3 \mu\text{m}$), dense particles, but SFD resulted in large ($\sim 8-10 \mu\text{m}$), porous particles. The fine particle fraction (FPF) of the spray freeze-dried powder was significantly better than that of the spray-dried powder, attributed to better aerodynamic properties. Powders collected from different stages of the cascade impactor were characterized, which confirmed the concept of aerodynamic particle size. Protein formulation played a major role in affecting the powder's aerosol performance, especially for the carbohydrate excipient of a high crystallization tendency.

Conclusions. Spray freeze drying, as opposed to spray drying, produced protein particles with light and porous characteristics, which offered powders with superior aerosol performance due to favorable aerodynamic properties.

KEY WORDS: spray freeze drying; spray drying; dispersibility; fine particle fraction; liquid impingement; cascade impaction; aerodynamic particle size.

INTRODUCTION

Powder production and handling has been an integral part of pharmaceutical processing because of the wide use of oral dosage forms. There are a few commonly used powder preparation methods including mechanical milling, precipitation, spray drying, and so on (1). Although not as widely explored (because stable oral formulations are yet to be developed), biopharmaceutical (protein) powders find increasing applications in dry powder inhalation and sustained drug delivery systems. In general, methods available for preparing protein powders are limited due to certain protein's sensitive nature to the processing environments. This is particularly true for preparing dry powder aerosols where the aerodynamic particle size ($<5 \mu\text{m}$) and the

size distribution are pivotal. Spray drying is probably so far the most popular method. Supercritical fluid antisolvent (2,3) and spray freeze drying (4) have recently emerged as promising techniques for producing powders for use in microencapsulation. However, the aerosol applications of these powders are yet to be explored. The purpose of this study was to test the feasibility of using spray freeze-dried protein powders for aerosolization.

The success of a dry powder inhalation product is based on the ease of powder dispersibility, which is mainly determined by the efficiency of inhalation devices and by the physical properties of the powder. Many physical characteristics affect the dispersibility of the powder, including the nature of the material, particle size/distribution, particle shape/morphology, and moisture content (5,6). All these properties affect the interparticle (cohesion) forces and/or the particle-surface (adhesion) forces. Increased interparticle cohesion reduces powder segregation, resulting in aggregated particles that may not enter the deep lung. Increased particle-surface adhesion decreases powder flowability and increases powder retention on all contact surfaces. However, even when particles are physically small enough ($<5 \mu\text{m}$), they are likely to be deposited on the wall of the respiratory tract on their way down to the alveolar regions of the lungs because inertial deposition is often the most dominating deposition mechanism. Particles with sufficient inertia can easily escape from the streamlines of air flow and deposit on the airway. Based on fluid dynamics, the aerodynamic diameter (D_a) of a particle (physical diameter D_s and density ρ_s) can be defined as $D_a = D_s \rho_s^{0.5}$. The significance of aerodynamic particle size lies in combining the influence of the particle's physical size and inertia. Assuming a light (low density) particle having the same physical size (D_s) as a heavy (high density) particle, the light particle will have a smaller aerodynamic size, i.e. more aerodynamically favorable, than the heavy particle; therefore, light particles are more likely to travel with air streamlines and reach in the deep lung for effective deposition. Changes in aerodynamic size for particles of the same composition and shape can be made by changing particle density, for example, from a solid sphere to a porous ball. This concept has been addressed recently by Edwards, et al. using a different technique for preparing large porous particles (7). The two techniques used in this study, spray drying and spray freeze drying, serve as a good example. We will examine the physical and aerosol properties of the powders prepared by these two methods.

MATERIALS AND METHODS

Materials

Proteins

Recombinant-derived humanized anti-IgE monoclonal antibody (146.5 kDa molecular weight) and recombinant human deoxyribonuclease (rhDNase) (32.7 kDa) were produced at Genentech, Inc. Both recombinant proteins contained carbohydrates. Excipient-free anti-IgE MAb and rhDNase solutions were prepared by ultrafiltration (UF) and diafiltration (DF) into a concentration of 50 g/L, and then appropriate amounts of a carbohydrate excipient were added to prepare a desired formulation. All protein solutions were filtered with a 0.22 μm filter before use.

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Carbohydrate Excipients

Mannitol, trehalose, and sucrose were obtained from Sigma and were used as supplied.

Methods

Spray Drying

Spray drying was performed using a Model 190 Buchi mini spray dryer (Brinkmann, Westbury, NY). Using compressed air from an in-house supply (~80 psi), a two-fluid nozzle (0.5 mm) atomized the protein solution. The air was filtered through a 0.22 μm Millidisk filter (Millipore, Bedford, MA) before entering the nozzle, and the flow rate was controlled by a variable area flow meter (Cole Parmer, Vernon Hills, Illinois, 150 mm). A peristaltic pump (1–100 rpm, Masterflex, Cole Parmer) pumped liquid protein feed to the nozzle using silicone tubing (3 mm ID). Cooling water was circulated through a jacket around the nozzle. Modifications to the original design included the replacement of the bag-filter unit with a vacuum cleaning unit (Model 005, VAC-U-MAX, Belleville, NJ) and relocation of the aspirator to the drying air input (7). The standard operating condition was: T_{inlet} (inlet air temperature) of 100–105°C, Q_{DA} (drying air flow rate) of 1000 L/min, Q_{AA} (atomizing air flow rate) of 1050 L/hr, and Q_{LF} (liquid feed rate) of 15 mL/min. This condition resulted in an T_{outlet} (outlet air temperature) of 50–55°C.

Spray Freeze Drying

A two-fluid nozzle (the same nozzle used in spray drying) or an ultrasonic nozzle (Soniteck) was used for atomization to spray the protein solution into a 3-L two-neck, round-bottom flask full of liquid nitrogen. The whole flask was submerged in liquid N₂ to ensure the system's low temperature. The liquid N₂ in the flask was agitated using a magnetic stirrer bar. Sprayed droplets froze upon contacting liquid N₂. The protein liquid was atomized using an atomizing air flow rate of 1050 L/hr. The liquid feed rate was 15 mL/min for air atomization and 5 mL/min for ultrasonic atomization. The spray of high-pressure air into liquid nitrogen resulted in liquid level lowering due to evaporation. This might result in material loss to the wall of the flask especially when a long atomization process (a large liquid volume to be sprayed) was used. Continuous addition of fresh liquid nitrogen into the flask would alleviate this problem. After spraying, the whole content in the flask was poured into a metal tray and placed in a lyophilizer (GT20) which had been pre-chilled to –50°C. After a hold period of one hour at –50°C, vacuum was applied to the chamber. The shelf temperature was increased to –25°C over a two-hour period and held for 40 hours. During secondary drying, the shelf temperature was increased to 20°C over a four-hour period and was held for another 20 hours.

Protein and Powder Characterizations

Scanning Electron Microscopy (SEM). Surface morphology of coated powder was examined using a Philips SEM system (Model 525M). Powder samples were mounted to a sample stub, and coated under a high vacuum (<0.05 mTorr) with a layer of 10 nm gold-platinum. All samples were scanned

at a voltage of 4.0 kV and their photographs were taken at two magnifications, 4,000 and 15,000.

Moisture Content. Moisture content of the protein powder was measured using a thermogravimetric analyzer (TGA 7, Perkin-Elmer) linked to a data station (Model 7700, Perkin Elmer). Samples (~5 mg) were loaded in aluminum pans and heated at 4°C/min under 30 mL/min N₂ gas purge. The moisture content was based on the loss in weight between room temperature and 150°C.

Particle Size Analysis. A Malvern laser defraction analyzer (Mastersizer-X) measured the particle size distribution of the spray-dried powder in a liquid suspension. The experimental procedure has been described elsewhere (9,10). Span was defined as $[D(v,90)-D(v,10)]/D(v,50)$, where $D(v,90)$, $D(v,10)$, and $D(v,50)$ were the respective diameters at 90, 10, and 50% cumulative volume.

Preparation of Blends. Before powder dispersion measurement, each powder was blended with a lactose carrier (200M, DMV) at the 10:1 (carrier:powder) weight ratio by mixing using a tumbling mixer (Turbula, Glen Mill) and sieving using a stainless steel sieve (250 μm). The blend was first mixed for 5 min and then sieved by tapping. Some clumps were gently pressed through the sieve to deagglomerate the particles. The same mixing and sieving procedures were repeated for the second time.

Powder Dispersion by Liquid Impingement. The dispersibility of each powder/carrier blend was assessed using the multiple-stage liquid impinger through a dry powder inhaler (Dryhaler, Dura Corp., San Diego, CA). All four stages were loaded with 25 mL water before experiment. Ten doses (10–20 mg each) of the blend sample were weighed out and loaded individually directly into the dose chamber of the device. The powder was dispersed at an inspiration rate of 60 L/min. The amount of protein deposited on the throat, four stages of the impinger, and the filter, as well as the amount retained in the device was assayed by measuring the UV absorbance at 280 nm using an absorptivity of 1.6 cm^{-1} (mg/mL)⁻¹. The percentage of the total dose collected on the third and the fourth stages and on the filter, representing the particles with aerodynamic diameters $\leq 6.4 \mu\text{m}$, was considered as the fine particle fraction (FPF).

Powder Dispersion by Cascade Impaction. The Anderson cascade impactor (8 stage 1 ACFM Non Variable Particle Size Sampler Mark II) was also used to determine the dispersibility of each powder/carrier blend through the same dry powder inhaler (Dryhaler). The eight metal plates of the impactor were coated with a thin layer of silicone grease to prevent particles from bouncing off the plates and becoming reentrained in the air stream. A preseparatory was attached to the top of the impactor to prevent large particles or aggregates from reaching the stages. The same throat piece that simulated the human throat used in liquid impingement was connected to the preseparatory. Ten doses (10–20 mg each) of the blend sample were weighed out and loaded individually directly into the dose chamber of the device and dispersed at an inspiration rate of 28.3 L/min for an inhalation time of 5 sec. After each determination the powders on each plate of the impactor was collected by rinsing

with deionized water. The protein concentration was assayed by measuring the UV absorbance at 280 nm using an absorptivity of $1.6 \text{ cm}^{-1}(\text{mg/mL})^{-1}$. The amount of protein deposited in the throat piece, the preseparator, and the device was also determined. The cutoff aerodynamic size ranges for Stage 0 to Stage 8 are 9.0–10, 5.8–9.0, 4.7–5.8, 3.3–4.7, 2.1–3.3, 1.1–2.1, 0.65–1.1, and 0.43–0.65 μm , respectively. Particles collected on the filter are smaller than 0.43 μm . The percentage of the total dose collected on the third stage and lower, representing particles with the aerodynamic diameter $\leq 5.8 \mu\text{m}$, was considered as the fine particle fraction.

Specific Surface Area. The specific surface area per unit weight of the powder samples was determined by the multipoint BET method from the adsorption of nitrogen gas at 77°C (Autosorb-3 Gas Sorption Analyzer, Quantachrome Corp.). All samples were outgassed at 25°C for 16 hrs.

X-ray Powder Diffraction (XRD). XRD measurements were conducted using a 35 kV \times 15 mA Rigaku (D/max-B, CuK α radiation) X-ray diffractometer at room temperature and humidity. Approximately 100 mg of powder (loaded onto the surface of a glass slide) was required for each measurement. Samples were scanned at 0.1 degrees/sec with 1 sec count time per increment. The range scanned was from 5 to 40 degrees.

RESULTS AND DISCUSSION

Physical Characteristics and Aerosol Performance of SFD vs SD Powders

Spray drying and spray freeze drying produced powders of different physical and aerosol dispersion properties (top part of Table 1) for both excipient-free rhDNase and anti-IgE MAb. The spray freeze-dried powders had larger median particle size, larger specific surface area, and higher fine particle fraction than the spray-dried powders. With the spray-drying condition used in this study, atomization resulted in droplets of approximately 10 μm in median diameter. The size of these droplets shrank to 3 μm upon water removal by hot air during drying.

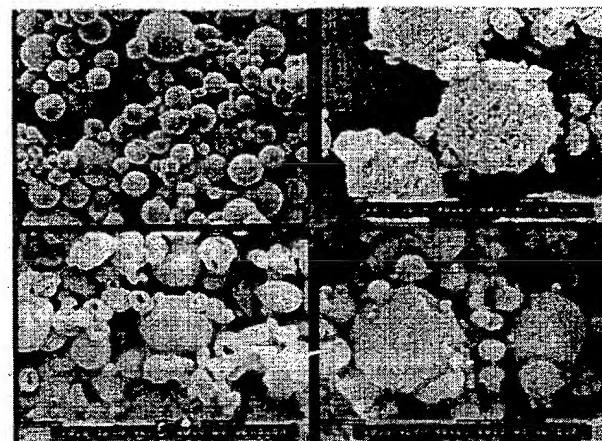


Fig. 1. SEMs for the four powders of excipient-free rhDNase spray-dried (top, left) and spray freeze-dried (top, right) and for anti-IgE MAb spray-dried (bottom, left) and spray freeze-dried (bottom, right).

Although the atomized droplets were spherical in shape, the shape of the dried particles might change depending on drying conditions and protein formulations (6). However, in the absence of hot air drying, atomized droplets during SFD maintained their spherical shape and size upon immediate freezing, and the subsequent drying process did not affect the shape and size either. Instead, the SFD process rendered particles porous. The significant increase (~40 times) in specific surface area for the SFD powder suggested its highly porous structure. SEMs of these powders (Fig. 1) confirm that spray-dried particles showed spherical but dimpled shapes and spray freeze-dried particles were spherical but porous. Assuming that the protein solid left in the porous structure accounted for 5% of droplet volume, the particle density of the spray freeze-dried powder would be reduced to approximately one-ninth of the density of the spray-dried particle which was determined to be 1.3 g/mL. Therefore, the aerodynamic particle size, $D_a = D_s \rho_s^{0.5}$, was calculated to be 2.7 μm for the spray freeze-dried powder and 3.5

Table 1. Physical and Aerosol Properties of Spray-Dried and Spray Freeze-Dried Powders of Two Proteins (rhDNase and anti-IgE Antibody)

Formulation	Method	Atomization ^a	Particle size (μm) ^b	Surface area (m^2/g)	FPF (%) ^c
Excipient-free rhDNase	SD	Air at Q_{AA} of 1050 L/hr	3.4(1.2)	3.4	46
	SFD		7.0(1.4)	121.2	70
Excipient-free anti-IgE antibody	SD	Air at Q_{AA} of 1050 L/hr	3.3(1.1)	2.8	27
	SFD		7.7(1.3)	127.7	50
Anti-IgE antibody: trehalose = 60:40	SFD	Ultrasound	32(2.4)	44.1	<10
	SFD	Air at Q_{AA} of 600 L/hr	19(1.6)	49.7	16
	SFD	Air at Q_{AA} of 1050 L/hr	5.9(1.2)	72.9	52

^a Spray drying and spray freeze drying conditions were described in the Methods section. Q_{AA} is the atomizing air flow rate.

^b Numbers in parenthesis represent the particle size distribution (span) defined in the Method Section.

^c Fine particle fraction was determined using liquid impingement for particles less than 6.4 μm .

μm for the spray-dried powder. The SFD process resulted in powders of a suitable aerodynamic particle size for inhalation.

The FPF of these four powders was determined using a multi-stage liquid impinger. Spray freeze-dried powders consistently outperformed spray-dried powders. The weight distribution of the powder deposited in each stage of the impinger is shown in Fig. 2a and b for excipient-free rhDNase and anti-IgE MAb. The result indicates that the increase in powder deposition in lower stages (Stages 3 and 4 and the filter) for the spray freeze-dried powders was due mainly to the decrease in powder deposition in the device and the throat. In addition, as far as inhalation to the deep lung, a significantly better dispersibility for particles of $<3 \mu\text{m}$ (Stage 4 and the filter) was observed for rhDNase, 53% vs. 15%, and for anti-IgE MAb, 20% vs. 11%, justifying the use of spray freeze-dried powders for aerosol applications.

Effect of Particle Size on Powder Dispersibility

Strong interparticle (between particles and/or the carrier particles) forces as well as adhesion to contacting surfaces prevented the powder from being fully dispersed. The plot of

aerodynamic diameter vs. cumulative percent undersize can be used to quantify the deviation of the aerosolized powder from complete dispersion. Such plots (Fig. 3a and b) are presented for pure spray-dried and spray freeze-dried anti-IgE MAb powders. Powder suspension in isopropanol followed by sonication may simulate a complete dispersion of a powder, and its physical

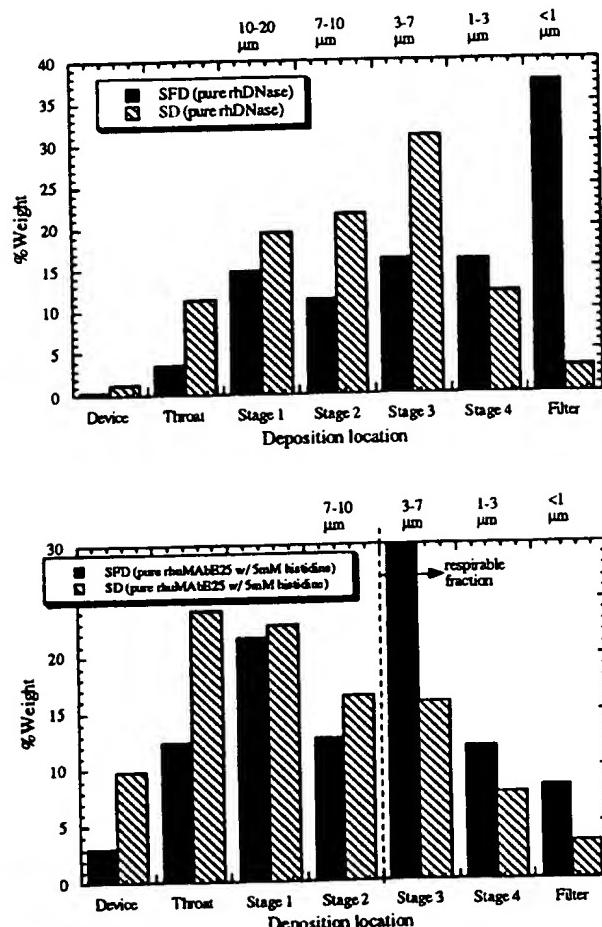


Fig. 2. Comparison of powder dispersion by liquid impingement between spray-dried and spray freeze-dried powders for excipient-free rhDNase (top) and anti-IgE MAb (bottom).

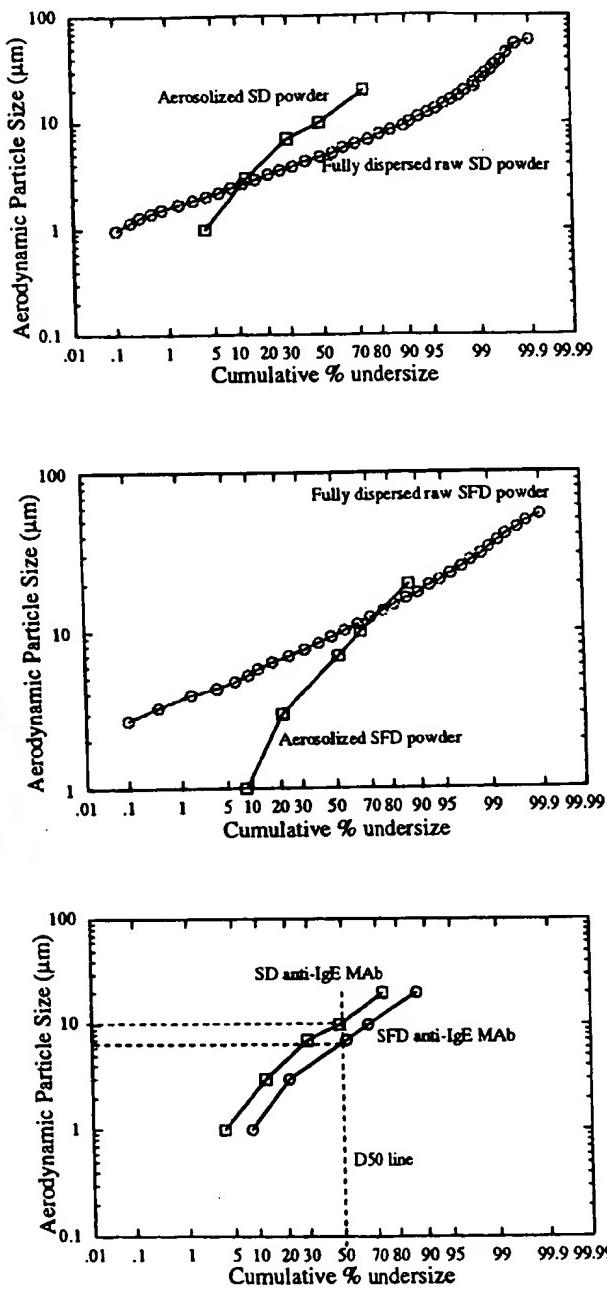


Fig. 3. The comparison between aerodynamic particle size distribution for the aerosolized powder (□) and the fully dispersed raw powder (○) based on liquid impingement data for the spray-dried anti-IgE MAb powder (top) and the spray freeze-dried anti-IgE MAb powder (middle). The mass median aerodynamic diameter for the aerosolized spray-dried (□) and spray freeze-dried (○) anti-IgE MAb powders is presented in (bottom).

particle size distribution could then be determined based on laser-based particle size analysis (for spherical particles in this case). In both cases, the aerosolized powder showed a sharper slope than the dispersed raw powder, suggesting a lower degree of dispersibility. However, the spray-dried and spray freeze-dried powders showed a similar dispersibility (similar slope in Fig. 3c) but their mass median aerodynamic diameters (MMAD) were 10 and 6.5 μm , respectively. These results suggest that the superior aerosol performance by the spray freeze-dried powder might be simply due to its smaller aerodynamic particle size despite its larger physical size.

Furthermore, spray freeze-dried powders (anti-IgE MAb:trehalose = 60:40) prepared by ultrasonic atomization and two-fluid atomization resulted in three different physical sizes (bottom part of Table 1). Ultrasonic atomization produced a powder of the largest physical size (32 μm) and the smallest surface area (44.1 m^2/g). Two-fluid atomization resulted in smaller particles, 19 μm (49.7 m^2/g) and 5.9 μm (72.9 m^2/g) corresponding to atomizing air flow rates of 600 and 1050 L/min. The FPF decreased significantly with increasing physical size. Powders with a preferred FPF (>30%) could only be produced using two-fluid atomization at an atomizing air flow rate of >1000 L/hr.

Aerodynamic Particle Size

To further confirm the concept of aerodynamic particle size, the powder's FPF was determined using a cascade impactor. Powders collected on Stage 3 (4.7–5.8 μm) and Stage 6 (1.1–2.1 μm) were examined using SEM (Fig. 4a-d). For particles collected on Stage 3, spray-dried particles (Fig. 4a) were mainly <5 μm , but some spray freeze-dried particles from the same stage (Fig. 4b) were larger than 10 μm in physical size. For particles collected on Stage 6, spray-dried particles (Fig. 4c) were smaller than 2 μm while some spray-freeze-dried particles were in the range of 4–5 μm . All this suggests that spray freeze drying produces large, porous particles which are aerodynamically favorable for aerosol delivery.

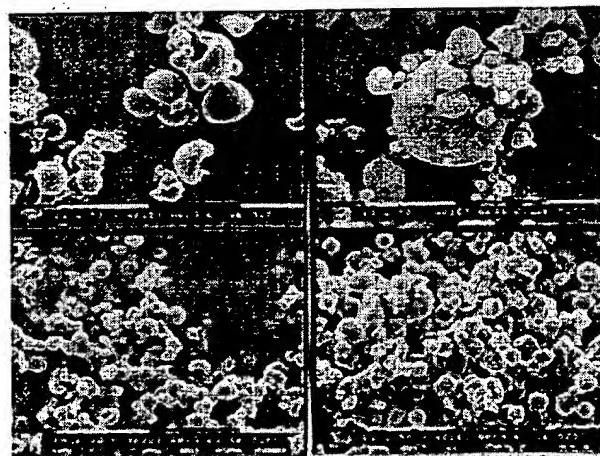


Fig. 4. SEMs for particles of 90/10 E25/mannitol collected in the Anderson cascade impactor from Stages 3 (4.7–5.8 μm) for the spray-dried powder (top, left) and the spray freeze-dried powder (top, right), and from Stage 6 (1.1–2.1 μm) for the spray-dried powder (bottom, left) and the spray freeze-dried powder (bottom, right).

Formulation Effect

Carbohydrate excipients tested here included trehalose, sucrose, and mannitol. After spray drying and SFD, the powder's physical size and FPF were determined (Table 2). Consistent with the case of excipient-free protein powders, spray freeze-dried powders outperformed spray-dried powders in aerosolization although their physical size were almost twice as large. However, there were a few exceptions: (i) 50/50 rhDNase/mannitol, (ii) 40/60 rhDNase/trehalose, and (iii) 60/20/20 E25/mannitol/trehalose.

In (i), the physical sizes of the two powders were close. SEM suggests mannitol crystallized in both the spray-dried (Fig. 5a) and the spray freeze-dried (Fig. 5b) powders (X-ray powder diffraction data not shown). Upon spray drying, larger, fused particles, which were formed as the result of mannitol crystals growing between particles, reduced the FPF significantly. Spray freeze-dried particles were highly deformed from spherical but maintained excellent aerosol performance. In Case (ii), the FPF (Table 2) of spray freeze-dried powder (Fig. 5d) was not better than that of the spray-dried powder (Fig. 5c). SEM analysis suggested that the spray freeze-dried particle lost the characteristics of porosity because of a high concentration of trehalose. In Case (iii), the spray-dried particle showed dimple morphology (Fig. 5e) which was consistent with our previous finding (6). The spray freeze-dried particle was highly deformed and showed a crystalline character (Fig. 5f) (X-ray powder

Table 2. Physical Size and Fine Particle Fraction of Spray-Dried and Spray Freeze-Dried Powders of rhDNase and Anti-IgE MAb in Formulation with Different Sugars

Formulation	Method	Median particle size (μm) ^a	Fine particle fraction (%) ^b
Excipient-free DNase	SD	3.4	46
	SFD	7.0	70
DNase/mannitol 50/50	SD	6.1	14
	SFD	7.7	67
DNase/trehalose 80/20	SD	2.9	29
	SFD	6.0	73
DNase/trehalose 60/40	SD	2.6	36
	SFD	7.1	56
DNase/trehalose 40/60	SD	3.2	20
	SFD	5.7	23
DNase/sucrose 60/40	SD	2.8	27
	SFD	6.3	56
Excipient-free anti-IgE MAb	SD	3.3	27
	SFD	7.7	50
Anti-IgE MAb/mannitol 90/10	SD	3.8	25
	SFD	8.0	45
Anti-IgE MAb/mannitol 80/20	SD	4.0	29
	SFD	11.0	40
Anti-IgE MAb/trehalose 60/40	SD	3.3	31
	SFD	5.9	52
Anti-IgE MAb/mannitol/trehalose 60/20/20	SD	3.6	19
	SFD	8.6	19

^a Determined by Malvern particle size analyzer.

^b Determined by multiple stage liquid impingement.

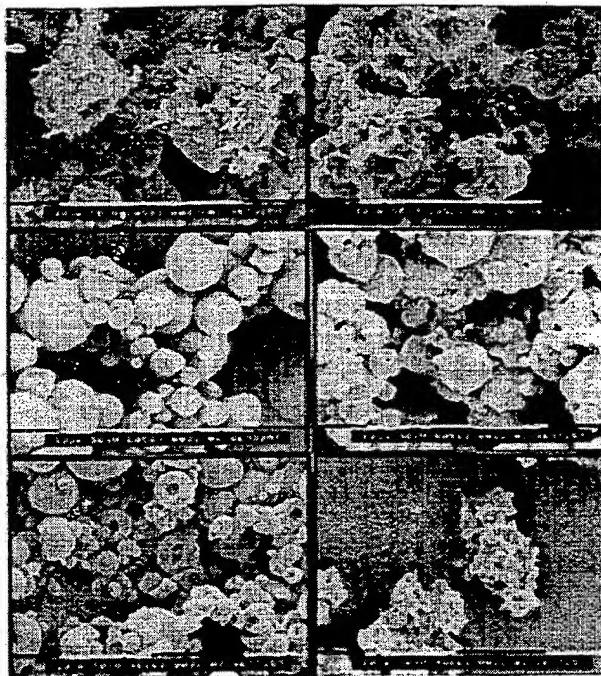


Fig. 5. SEMs for the spray-dried powder and the spray freeze-dried powder for three formulations: 50/50 rhDNase/mannitol (top), 40/60 rhDNase/trehalose (middle), and 60/20/20 E25/mannitol/trehalose (bottom).

diffraction data not shown). It appears that the spray freeze-drying process promoted the tendency of mannitol crystallization, which might affect protein biochemical stability. An investigation of this subject is ongoing.

Process Comparison

The two processes as compared in Table 3 suggest that spray drying is a relatively easy, fast, and convenient process, but the SFD process is more time-consuming and complex in terms of operation. The operating cost is higher for spray freeze drying because of the additional freezing process and the more expensive lyophilization process. The scalability of the two

Table 3. Process Comparisons Between Spray Drying and Spray Freeze Drying

Factors	Spray Drying	Spray Freeze Drying
Operation	Easy, fast, convenient	Time consuming, Inconvenient to handle liquid N ₂
Scalability	Straightforward	Comparable to SD but more complicated due to freezing by liquid N ₂
Operation cost	Low	High
Yield	50–70%	>95%
Aerosolization	Good	Excellent (at least 50% better)

processes is comparable because both are limited by the speed of atomization. The biggest advantages offered by spray freeze drying are the high production yield (>95%) and the powder's superior aerosol performance which may eventually make spray freeze drying a more economical process than spray drying. However, the process of spray freeze drying might involve more stressful events which might affect protein's stability than the spray drying process. Therefore, continuing investigation on spray freeze drying as an alternative protein aerosol powder technique will be focused on biochemical aspects.

CONCLUSIONS

We demonstrated that spray freeze drying is a feasible technique for preparing protein aerosol powders. The spray freeze-dried powders showed much better aerosol performance than the spray-dried powders, which was attributed to better aerodynamic properties as the result of the powder's large, porous characteristics. However, protein formulation played an important role. Excipients that crystallized or tended to coalesce deteriorated the aerodynamic properties of the spray freeze-dried powder. Overall, spray freeze drying is a more efficient process in terms of product recovery and product quality. The future work will be focused on investigating the process effect on protein stability.

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REFERENCES

1. E. G. Rippie. Powders. In: E. W. Martin (ed), *Remington's Pharmaceutical Sciences*, 17th ed., pp. 1585–1602 (1985).
2. S.-D. Yeo, G.-B. Lim, P. G. Debenedetti, and H. Bernstein. Formation of microparticulate protein powders using a supercritical fluid antisolvent. *Biotechn. Bioeng.* 41:341–346 (1993).
3. M. A. Winters, B. L. Knutson, P. G. Debenedetti, H. G. Sparks, T. M. Przybycien, C. L. Stevenson, and S. J. Prestrelski. Precipitation of proteins in supercritical carbon dioxide. *J. Pharm. Sci.* 85:586–594 (1996).
4. W. R. Gombotz and L. R. Brown. Process for producing small particles of biologically active molecules. International Patent Application PCT/US90/02421 (1990).
5. A. J. Hickey, N. M. Concessio, M. M. Van Oot, and R. M. Platz. Factors influencing the dispersion of dry powders as aerosols. *Pharm. Tech.* 18:58–82 (1994).
6. Y.-F. Maa, H. R. Costantino, P.-A. Nguyen, and C. C. Hsu. The effect of operating and formulation variables on the morphology of spray-dried protein particles. *Pharm. Dev. Tech.* 2:213–223 (1997).
7. D. A. Edwards, J. Hanes, G. Caponetti, J. Hrkach, A. Ben-Jebria, M. L. Eskew, J. Mintzes, D. Deaver, N. Lotan, and R. Langer. Large porous particles for pulmonary drug delivery. *Science* 276:1868–1871 (1997).
8. Y.-F. Maa, P.-A. Nguyen, K. Sit, and C. C. Hsu. Spray-drying performance of bench-top spray dryer for protein aerosol powder preparation. *Biotechn. Bioeng.* in press.
9. Y. F. Maa, P. A. Nguyen, J. D. Andya, N. Dasovich, T. D. Sweeney, S. J. Shire, and C. C. Hsu. Effect of spray drying and subsequent processing conditions on residual moisture content and physical/biochemical stability of protein inhalation powders. *Pharm. Res.* 15:768–775 (1998).
10. Y. F. Maa, P. A. Nguyen, and C. Hsu. Spray drying of air-liquid interface sensitive recombinant human growth hormone. *J. Pharm. Sci.* 87:152–159 (1998).